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STUDIES ON IMMUNITY IN EXPERIMENTAL SYPHILIS*†‡

II. *TREPONEMA PALLIDUM* IMMOBILIZATION (TPI) ANTIBODY AND THE IMMUNE RESPONSE

BY

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The classic studies of Chesney (1926), Magnuson and Rosenau (1948), Magnuson, Roesnau, and Clark (1949), and Turner and Nelson (1950) have shown that acquired immunity in experimental syphilis develops in the same way as other infectious diseases. Rabbits injected intratesticularly or intracutaneously with virulent *T. pallidum* begin to develop a relative immunity within 3 weeks after the "immunizing infection". As the infection progresses, resistance to challenge increases, reaching a maximum within 3 months and remaining high for the duration of the untreated animal's life. If the "immunizing infection" is terminated with curative penicillin in less than 3 months, at a time when resistance is not fully developed, the animal can be consistently re-infected upon challenge. However, if therapy is instituted after allowing the infection to persist 3 months or longer, at a time when the immune response is at its maximum, the high degree of resistance to challenge lasts for months and perhaps years after treatment. The extent of this immunity is relative, depending upon the size of the infective dose, the duration of the original infection, and the host reaction to infection and challenge.

The mechanism whereby immunity to *T. pallidum* develops is poorly understood. Previous studies attempting to relate humoral antibody to acquired resistance have shown that neither reagin, Reiter protein complement-fixation (RPCF), nor *Treponema pallidum* complement-fixation (TPCF) antibody is associated *per se* with its development (Eagle and Fleischman, 1948; Turner and Nelson, 1950; Gelperin, 1951; McLeod, 1952; Miller, Whang, and Fazzan, 1963). On the other hand, studies designed to determine the relationship of *Treponema pallidum* immobilization (TPI) antibody to the immune response has led to the formation of two divergent concepts. Turner and Nelson (1950) have presented evidence which they feel suggests a direct relationship. Rabbits were infected with large numbers of treponemes and then given penicillin therapy 3 weeks, 2 months, and 6 months later. Upon re-inoculation, a direct correlation was found between the level of immobilizing antibody in serum and the degree of resistance to re-infection. They also found a delayed anamnestic TPI-antibody response 3 to 4 weeks after the challenge, which coincided with the development of partial immunity.

In contrast to these studies, Magnuson, Thompson and McLeod (1951) found only a crude correlation between TPI antibody and the degree of immunity in rabbits challenged 6 weeks after curative penicillin therapy. If, however, treatment was delayed 12 to 48 weeks, there was little or no correlation. In addition, the degree of resistance could not be related to an anamnestic TPI antibody response, and in some instances the latter failed to occur among immune animals after challenge. McLeod and Magnuson (1953) further showed that rabbits with

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immobilizing antibody levels after the injection of killed, virulent *T. pallidum* could be infected with minimal doses of treponemes. Thus, these investigators concluded that TPI antibody plays either no role or a limited role in the development of resistance and that other factors, possibly associated with a tissue response, may be of major importance.

In an effort to clarify the relationship of TPI antibody to the immune response, rabbit antibody levels were correlated with the development of resistance, with its persistence following curative penicillin therapy, and with the results of challenge 48 weeks after treatment. Reagin and RPCF antibody, known to have no relationship *per se* to the development of immunity, were also measured.

Material

Ten adult, Dutch, male rabbits with non-reactive VDRL, RPCF, and TPI tests were selected and housed in individual cages at a temperature of 68-70°F.

Methods

The methods for the production and demonstration of relative immunity to *T. pallidum* in rabbits is shown in Fig. 1.

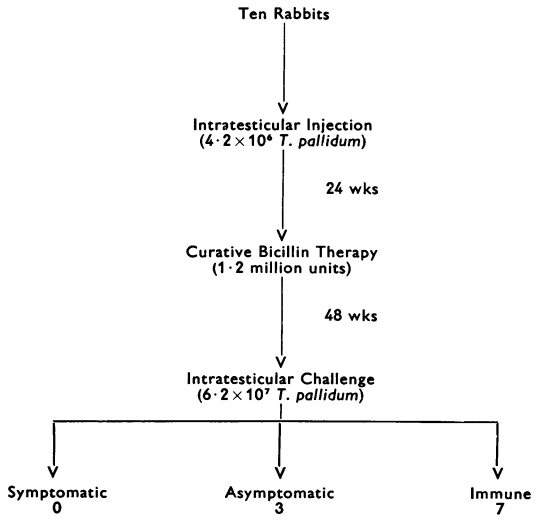

FIG. 1.—Production and demonstration of relative immunity in experimental syphilis.

The animals were inoculated intratesticularly with a total of $4 \cdot 2 \times 10^6$ virulent *T. pallidum*, as described previously by Miller, Whang, and Fazzan (1963), and each animal developed dark-field positive lesions in an average time of 24·3 days after infection. 24 weeks after the initial infection, at a time when immunity had reached a high level, the animals were injected intramuscularly with a single curative dose of 1·2 million units benzathine penicillin G (Bicillin). They were challenged intratesticularly with $6 \cdot 2 \times 10^7$ virulent *T. pallidum* 48 weeks later, and then examined during a 12-week period for the development of dark-field positive lesions.

As a control of infectivity, two rabbits were infected intratesticularly with the challenge inoculum. The immune status of the rabbits measured by popliteal lymph-node and testicular transfer, as well as their reagin, RPCF, and TPI antibody responses, were determined according to the methods previously described (Miller, Whang, and Fazzan, 1963).

Results

Clinical Response to Challenge

None of the ten rabbits developed symptomatic infection after the challenge with virulent *T. pallidum* as indicated by the absence of dark-field positive lesions. Three of the animals were asymptomatic (partially immune) and seven were considered to be immune (Fig. 1). The two control rabbits injected intratesticularly as a test of the infectivity of the challenge inoculum developed dark-field positive lesions 14 days after the inoculation. These results confirm the studies of Magnuson, Rosenau, and Clark (1949), who observed that a high degree of acquired resistance persists among immune animals for at least 48 weeks after curative therapy.

Serological Response to the Development and Persistence of Immunity and to the Challenge

The average TPI antibody titres of both the immune and asymptomatically-infected rabbits during the development of acquired resistance, after cure, and after challenge are shown in Fig. 2 (opposite). As the initial infection progresses and relative immunity increases, there is a corresponding increase in TPI titre among both groups of animals. After curative therapy and up to the time of challenge, at a time when immunity is presumably at a relatively high level, the average TPI titres decreased slightly, the sera from some animals exhibiting non-reactivity. In contrast to the studies of Magnuson, Thompson, and McLeod (1951), the most significant finding was that 9 days after challenge an anamnestic TPI antibody response occurred among *all* the animals in both the immune and asymptomatic groups (Table I, p. 202). The peak level of immobilizing antibody after challenge was correlated closely with the time in which dark-field positive lesions would have developed had the animals been not immune or partially immune. Although the average titres after challenge convey the impression that significantly greater TPI

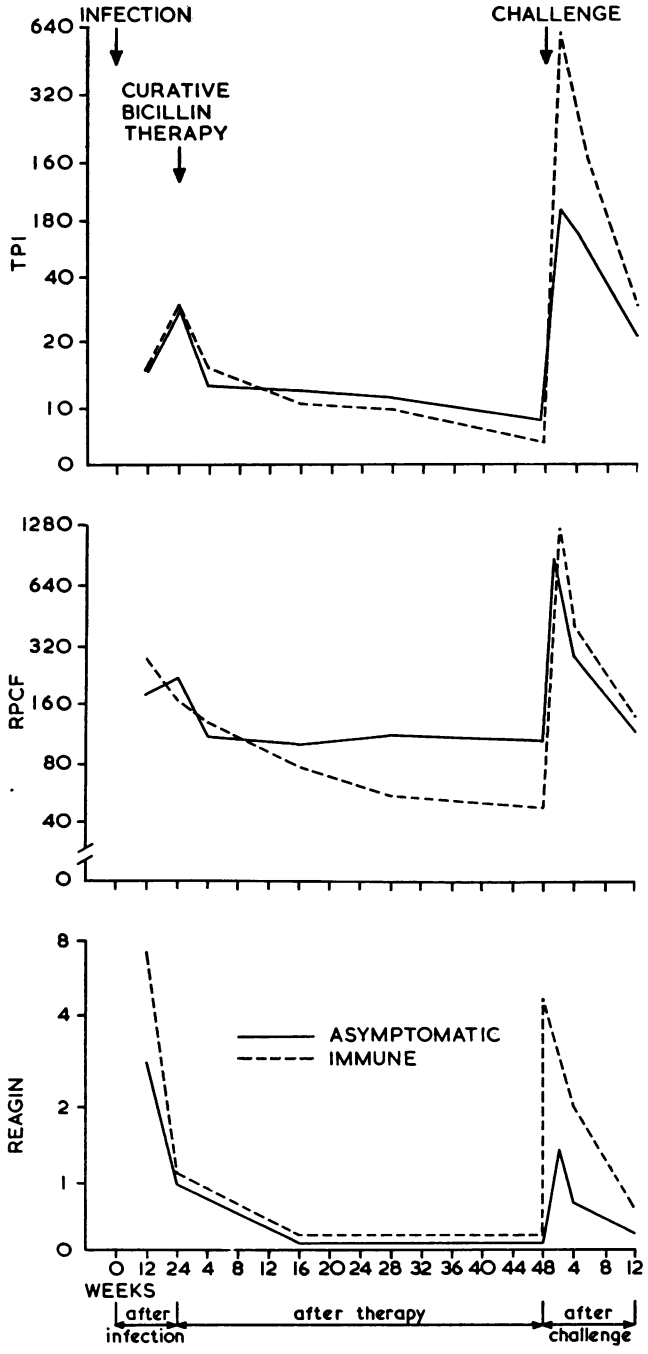

FIG. 2.—TPI and RPCF antibody titres and Reagin titres in rabbits experimentally infected, cured, and subsequently challenged with virulent *T. pallidum*.

titres occurred in the immune than in the asymptomatic group (Fig. 2), the response of the individual animals indicates that this is not a true correlation. Four of the seven immune animals showed peak titres similar to those observed in the asymptomatic group. Moreover, it is interesting to note that four of the immune animals exhibited non-reactive TPI tests just before challenge, whereas the asymptomatic animals all showed some degree of reactivity (Table I).

The RPCF and TPI antibody patterns were similar for both groups of animals although the RPCF titres were higher (Fig. 2). All animals exhibited significant increases in RPCF titre after challenge, the peak level appearing at approximately the same time as immunity to symptomatic infection (Table II); no differences in the individual or average peak titres were observed among the immune and asymptomatically-infected rabbits. All the animals in both groups exhibited some degree of RPCF reactivity just before challenge whereas the sera of four of the seven immune animals lacked immobilizing antibody (Table II, overleaf).

Reagin levels, as opposed to the TPI and RPCF antibody response, declined sharply as maximum acquired resistance developed and continued to drop after curative therapy until all the animals in both groups were non-reactive just before challenge (Fig. 2). Although the patterns of the average reagin, TPI, and RPCF anamnestic responses after challenge were similar, the reagin response was lower. The average peak reagin titres appeared greater among the immune than among the asymptomatically-infected rabbits. However, as in the case of the TPI antibody response, the lack of true correlation is shown by the response of the individual rabbits; four of the seven in the immune group exhibited the same increase in titre as those in the asymptomatic group (Table III, overleaf).

Discussion

Other investigators have suggested that TPI antibody plays an important role in the development of acquired resistance to *Treponema pallidum* (Turner and Nelson, 1950). The present studies, however, indicate that such conclusions (based upon an increase in antibody titre as immunity develops, persistence of a high titre after curative therapy, and an anamnestic response after challenge) are, at best, premature. Similar evidence may be employed to relate RPCF antibody to immunity, despite the fact that high titres fail to protect rabbits against symptomatic infection with *T. pallidum* (Miller, Whang, and Fazzan, 1963). The occurrence of a "booster" reagin response after challenge casts further doubt

TABLE I

TPI ANTIBODY TITRES IN RABBITS EXPERIMENTALLY INFECTED, CURED, AND SUBSEQUENTLY CHALLENGED WITH VIRULENT *T. PALLIDUM*

Status of Immunity		TPI Titres (Reciprocal)								
		24 wks after Infection		Weeks after Bicillin Therapy					Weeks after Challenge	
				4	16	28	48		1·3	4	12
Asymptomatic	49	20	Curative Bicillin Therapy	10	10	—	<10	Challenge	40	20	10
	57	40		20	10	10	<10		80	40	40
	55	40		10	10	10	10		160	160	20
Immune	42	80		40	40	10	20		1280	640	80
	46	10		10	N	10	<10		640	320	40
	52	20		10	10	10	10		640	160	40
	58	20		10	10	N	N		160	20	20
	59	40		20	10	N	<10		160	80	10
	51	20		10	N	10	N		80	40	—
	60	40		20	10	20	N		40	10	10

TABLE II

RPCF ANTIBODY TITRES IN RABBITS EXPERIMENTALLY INFECTED, CURED, AND SUBSEQUENTLY CHALLENGED WITH VIRULENT *T. PALLIDUM*

Status of Immunity		RPCF Titres (Reciprocal)								
		24 wks after Infection		Weeks after Bicillin Therapy					Weeks after Challenge	
				4	16	28	48		1·3	4	12
Asymptomatic	49	80	Curative Bicillin Therapy	80	40	—	40	Challenge	640	160	80
	57	320		160	80	80	40		640	320	160
	55	160		160	160	160	160		640	320	160
Immune	42	80		40	40	40	20		640	160	80
	46	160		80	80	80	40		640	320	80
	52	320		320	160	80	80		640	640	320
	58	160		160	40	20	20		640	160	80
	59	160		160	80	80	80		640	320	160
	51	160		80	80	40	40		160	160	—
	60	160		160	80	40	40		1280	640	320

upon this type of evidence, inasmuch as reagin *per se* is not related to immunity (Eagle and Fleischman, 1948).

The available data do not exclude the possibility that other antibodies stimulated by *T. pallidum* or its components may be related to the development of acquired resistance. In addition, cellular antibody, either fixed or soluble, and capable of destroying treponemes *in vivo* may play a significant part. The latter possibility is suggested by the fact that, within the popliteal lymph nodes of rabbits, *T. pallidum* is converted to a relatively avirulent form, as demonstrated by its failure to produce overt clinical manifestations; the treponemes persist in this latent form for the remainder of the untreated animal's life. Further evidence for this type of alternate or additional cellular mechanism stems from the observation that, although treponemicidal antibodies appear after injection of rabbits with killed *T. pallida*, immunity fails to develop. All studies to date indicate that the complete, intact virulent treponeme is essential for the development of the immune response.

Summary

(1) Ten rabbits were "immunized" with $4 \cdot 2 \times 10^6$ virulent *Treponema pallidum*, "cured" with benzathine penicillin G (Bicillin) 24 weeks

TABLE III

REAGIN TITRES IN RABBITS EXPERIMENTALLY INFECTED, CURED, AND SUBSEQUENTLY CHALLENGED WITH VIRULENT *T. PALLIDUM*

Status of Immunity		Reagin Titres (Reciprocal)									
		24 wks after Infection		Weeks after Bicillin Therapy					Weeks after Challenge		
				4	16	28	48		1·3	4	12
Asymptomatic	49	1	Curative Bicillin Therapy	1	N	N	N	Challenge	2	1	<1
	57	1		N	N	N	N		1	<1	N
	55	1		1	N	N	N		2	<1	N
Immune	42	1		1	N	N	N		2	<1	N
	46	1		N	N	N	N		8	2	<1
	52	1		1	N	N	N		4	2	<1
	58	2		1	1	<1	N		16	8	2
	59	1		N	N	N	N		2	1	N
	51	1		N	N	N	N		1	N	—
	60	1		N	N	N	N		2	1	N

later, and "challenged" with $6{\cdot}2 \times 10^7$ treponemes 48 weeks after treatment. Three of the animals developed asymptomatic infection while seven were immune to challenge.

(2) Both TPI and RPCF antibody increased as immunity to *T. pallidum* increased, whereas the reagin levels declined sharply during the development of maximum acquired resistance.

(3) Anamnestic TPI, RPCF, and reagin responses to challenge occurred among both the immune and partially-immune rabbits. Peak titres correlated closely with the time in which symptomatic infection would have developed had the animals not been resistant.

(4) No significant differences were observed in TPI, RPCF, or reagin responses after the challenge of individual immune rabbits and those asymptomatically infected.

(5) The evidence relating TPI antibody to immunity is inconclusive.

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II. Immobilisation de l'anticorps *T. pallidum* et réponse immunologique

RÉSUMÉ

(1) 24 semaines après "immunisation" par $4{,}2 \times 10^6$ *T. pallidum* virulent, dix lapins furent "guéris" par la benzathine pénicilline G (Bicillin), et 48 semaines plus tard ils furent soumis à l'attaque de $6{,}2 \times 10^7$ *T. pallidum*. Trois animaux eurent une infection asymptomatique et les autres furent immuns à l'attaque.

(2) Les anticorps T.P.I. et R.P.C.F. augmentèrent avec l'immunité contre *T. pallidum*, tandis que les taux de réagine tombèrent subitement pendant l'acquisition de la résistance.

(3) Parmi tous les animaux il y eut des réponses à l'attaque. Les titres les plus élevés se déclaraient au moment ou les symptômes d'infection se seraient développés chez les animaux non-immunisés.

(4) On n'observa aucune différence significative entres les réponses de T.P.I., R.P.C.F., ou réagine après l'attaque des individus immuns et celle des lapins infectés sans symptômes.

(5) L'association de l'anticorps T.P.I. et de l'immunité est encore inconnue.